

WHAT IS CLAIMED IS:

1. A method of screening candidate eukaryotic nucleic acid for one or more nucleic acid sequence encoding a signal sequence and/or a transmembrane sequence comprising:

- 5 a) providing a bacterial cell;
- b) contacting the bacterial cell with at least one plasmid comprising a candidate eukaryotic nucleic acid segment and a marker gene comprising a mutation in a region comprising a signal sequence and/or a transmembrane sequence of the marker gene; and
- 10 c) screening for function of the marker gene;

wherein function of the marker gene indicates that the candidate nucleic acid segment comprises a sequence that encodes a signal sequence and/or a transmembrane sequence.

2. The method of claim 1, wherein the nucleic acid is invertebrate nucleic acid.

3. The method of claim 2, wherein the invertebrate nucleic acid is fly nucleic acid.

4. The method of claim 2, wherein the invertebrate nucleic acid is *C. elegans* nucleic acid.

5. The method of claim 1, wherein the nucleic acid is vertebrate nucleic acid.

6. The method of claim 5, wherein the vertebrate nucleic acid is amphibian nucleic acid.

7. The method of claim 6, wherein said amphibian nucleic acid is frog nucleic acid.

8. The method of claim 5, wherein the vertebrate nucleic acid is reptile nucleic acid.

9. The method of claim 5, wherein said vertebrate nucleic acid is avian nucleic acid.

10. The method of claim 5, wherein the vertebrate nucleic acid is mammalian nucleic acid.
11. The method of claim 10, wherein the mammalian nucleic acid is mouse nucleic acid.
12. The method of claim 10, wherein the mammalian nucleic acid is human nucleic acid.
13. The method of claim 1, wherein the nucleic acid is fat cell nucleic acid.
14. The method of claim 12, wherein the nucleic acid is cancer cell nucleic acid.
15. The method of claim 14, wherein the cancer cell is obtained from a tumor or metastasis.
16. The method of claim 14, wherein the cancer cell is from an immortal cancer cell line.
17. The method of claim 14, wherein the cancer cell nucleic acid is breast cancer nucleic acid, hematological cancer nucleic acid, thyroid cancer nucleic acid, melanoma nucleic acid, T-cell cancer nucleic acid, B-cell cancer nucleic acid, ovarian cancer nucleic acid, pancreatic cancer nucleic acid, prostate cancer nucleic acid, colon cancer nucleic acid, bladder cancer nucleic acid, lung cancer nucleic acid, liver cancer nucleic acid, stomach cancer nucleic acid, testicular cancer nucleic acid, uterine cancer nucleic acid, brain cancer nucleic acid, lymphatic cancer nucleic acid, skin cancer nucleic acid, bone cancer nucleic acid, kidney cancer nucleic acid, rectal cancer nucleic acid, sarcoma nucleic acid, pituitary cancer nucleic acid, lipoma nucleic acid, adrenalcarcinoma nucleic acid; or nerve cell cancer nucleic acid.

18. The method of claim 17, wherein the cancer cell nucleic acid is breast cancer nucleic acid.

5 19. The method of claim 18, wherein the breast cancer cell nucleic acid is breast cancer cell line nucleic acid.

20. The method of claim 19, wherein the breast cancer cell line is an immortalized breast cancer cell line.

10 21. The method of claim 19, wherein the breast cancer cell line nucleic acid is MCF7 nucleic acid, SKBR-3 nucleic acid, MDA-MB-231 nucleic acid, MCF6 nucleic acid, T47D nucleic acid, or MDA-MB-435 nucleic acid.

15 22. The method of claim 18, wherein the breast cancer cell nucleic acid is a breast cancer sample.

23. The method of claim 1, wherein the nucleic acid is cultured cell nucleic acid.

20 24. The method of claim 1, wherein the nucleic acid is plant nucleic acid.

25 25. The method of claim 24, wherein the nucleic acid is corn, wheat, tobacco, arabidopsis, soybean, rice, or canola nucleic acid.

26. The method of claim 1, wherein the marker gene is further defined as a selectable marker gene comprising a mutation in a region comprising a signal sequence and/or a transmembrane sequence of the marker gene, and screening for function of the marker gene is further defined as assaying for survival of the cell or its progeny cells on the selectable media.

27. The method of claim 26, wherein survival of the cell or its progeny on selectable media indicates that the candidate nucleic acid sequence encodes a polypeptide comprising a signal sequence and/or a transmembrane sequence.

5 28. The method of claim 1, further comprising isolating at least one nucleic acid segment comprising a nucleic acid sequence encoding a polypeptide comprising a signal sequence and/or a transmembrane sequence from the candidate nucleic acid.

10 29. The method of claim 28, further defined as comprising isolating a plurality of nucleic acid segments comprising sequences encoding a polypeptide comprising a signal sequence and/or a transmembrane sequence from the candidate nucleic acid.

30. The method of claim 28, further comprising identifying at least one isolated nucleic acid segment.

15 31. The method of claim 30, wherein identifying comprises sequencing the nucleic acid sequence.

20 32. The method of claim 30, wherein identifying comprises expressing the nucleic acid sequence and identifying any polypeptides expressed.

33. The method of claim 32, wherein said identifying the polypeptides expressed is by using antibodies.

25 34. The method of claim 33, wherein the antibodies are prepared by phage display.

35. The method of claim 30, wherein identifying further comprises a cell-based assay.

30 36. The method of claim 30, wherein identifying further comprises a biochemistry-based assay.

37. The method of claim 28, further comprising characterization of at least one isolated nucleic acid segment.

38. The method of claim 37, further defined as comprising characterization of a plurality of isolated nucleic acid segments.

39. The method of claim 37, wherein the characterization comprises microarray analysis.

40. The method of claim 37, wherein the characterization comprises Northern blot analysis.

41. The method of claim 37, wherein the characterization comprises RT-PCR analysis.

42. The method of claim 37, wherein the characterization comprises expression of a polypeptide encoded by at least one candidate nucleic acid segment.

43. The method of claim 42, further defined as comprising analysis of function of the polypeptide.

44. The method of claim 40 further defined as comprising determining of antigenicity of the polypeptide.

45. The method of claim 37, wherein the characterization comprises determining whether the nucleic acid sequence or any polypeptide it encodes is an indicator of a disease, state of physiological condition, or other condition.

46. The method of claim 45, wherein the characterization comprises determining whether the isolated nucleic acid sequence or any polypeptide it encodes is an indicator of a disease.

47. The method of claim 46, wherein the disease is an endocrine disease, a renal disease, a cardiovascular disease, a rheumatologic disease, a hematological disease, a neurological disease, oncological, pulmonary, or a gastrointestinal disease.

5

48. The method of claim 47, wherein the disease is cancer, Alzheimer's disease, osteoporosis, coronary artery disease, congestive heart failure, stroke, or diabetes.

49. The method of claim 48, wherein the disease is cancer.

10

50. The method of claim 45, wherein the characterization comprises determining whether the isolated nucleic acid segment or any polypeptide it encodes is an indicator of a physiological condition.

15

51. The method of claim 50, wherein the state of physiological condition is a state of fat metabolism.

20

52. The method of claim 45, wherein characterization is further defined as determining whether the nucleic acid sequence or any polypeptide it encodes is an indicator that a subject has a disease, state of physiological condition, or other condition.

25

53. The method of claim 45, wherein characterization is further defined as determining whether the nucleic acid sequence or any polypeptide it encodes is an indicator that a subject has a propensity for a disease, state of physiological condition, or other condition.

30

54. The method of claim 45, further comprising determining that the nucleic acid sequence or any polypeptide it encodes is an indicator of a disease, state of physiological condition, or other condition.

55. The method of claim 54, further comprising assaying a subject for the nucleic acid sequence or any polypeptide it encodes to determine whether the subject has or has a propensity for a disease, state of physiological condition, or other condition.
- 5 56. The method of claim 55, further comprising determining that the subject has or has a propensity for a disease, state of physiological condition, or other condition.
57. The method of claim 1, wherein the bacterial cell is a gram negative bacterial cell.
- 10 58. The method of claim 1, wherein the bacterial cell is an *Acetobacter* cell, an *Acinetobacter* cell, a *Bacillus* cell, a *Brevibacterium* cell, a *Campylobacter* cell, a *Citrobacter* cell, a *Clostridium* cell, a *Corynebacterium* cell, an *Enterobacter* cell, an *E. coli* cell, a *Heliobacter* cell, a *Klebsiella* cell, a *Lactobacillus* cell, a *Leuconostoc* cell, a *Micrococcus* cell, a *Pseudomonas* cell, a *Staphylococcus* cell, a *Streptococcus* cell, a
- 15 *Thiobacillus* cell or a *Vibrio* cell.
59. The method of claim 58, wherein the bacterial cell is an *E. coli* cell.
60. The method of claim 58, wherein the bacterial cell is a *B. subtilis* cell.
- 20 61. The method of claim 58, wherein the bacterial cell is a *B. thuringiensis* cell.
62. The method of claim 58, wherein the bacterial cell is a *B. stearothermophilus* cell.
- 25 63. The method of claim 58, wherein the bacterial cell is a *B. licheniformis* cell.
64. The method of claim 1, where the marker gene is a screenable marker gene.
65. The method of claim 64, wherein the screenable marker gene is detectable by
- 30 fluorescence methods, colorimetric methods, radioactive, or enzymatic methods.

66. The method of claim 64, wherein the marker gene is a fluorescent protein gene or a beta-galactosidase gene.

67. The method of claim 1, where the marker gene is a scorable marker gene.

68. The method of claim 67, wherein the scorable marker gene is detectable by fluorescence methods, colorimetric methods, radioactive, or enzymatic methods.

69. The method of claim 1, where the marker gene is a measurable marker gene.

70. The method of claim 69, wherein the measurable marker gene is detectable by fluorescence methods, colorimetric methods, radioactive, or enzymatic methods.

71. The method of claim 1, where the marker gene is a selectable marker gene.

72. The method of claim 71, wherein the marker gene is an antibiotic resistance gene, a multidrug resistance gene, an herbicide resistance gene, or a toxin resistance gene.

73. The method of claim 71, where the marker gene is an antibiotic resistance gene.

74. The method of claim 73, where the antibiotic resistance gene is a beta-lactamase gene.

75. The method of claim 73, where the antibiotic resistance gene is an ampicillin-resistance gene, a penicillin-resistance gene, a cephalosporin-resistance gene, an oxacephem-resistance gene, a carbapenem-resistance gene, or a monobactam-resistance gene.

76. The method of claim 75, where the beta-lactamase gene is an ampicillin-resistance gene.

77. The method of claim 76, wherein the screening process comprises growth selection on selective media.

78. The method of claim 1, wherein the mutation is a deletion in the signal sequence of said marker gene.

79. The method of claim 1, wherein the mutation is a deletion of the entire signal sequence of said marker gene.

80. The method of claim 1, wherein the mutation is an insertion in the signal sequence of said marker gene.

81. The method of claim 1, wherein the mutation is a frameshift mutation in the signal sequence of said marker gene

82. The method of claim 1, wherein the mutation is a truncation of the signal sequence of said marker gene.

83. The method of claim 1, wherein the bacterial cell comprises a second marker gene.

84. The method of claim 83, wherein the second marker gene is a kanamycin resistance gene.

85. The method of claim 1, wherein the candidate nucleic acid is DNA.

86. The method of claim 85, wherein the candidate DNA is comprised in a DNA library.

87. The method of claim 86, wherein the DNA library is a genomic DNA library.

88. The method of claim 86, wherein the DNA library is an oligonucleotide library.
89. The method of claim 86, wherein the DNA library is a cDNA library.
- 5 90. The method of claim 86, wherein at least two members of the library are screened.
91. The method of claim 86, wherein at least 10 members of the library are screened.
92. The method of claim 86, wherein at least 100 members of the library are screened.
- 10 93. The method of claim 86, wherein at least 1000 members of the library are screened.
94. The method of claim 86, wherein at least 10,000 members of the library are screened.
- 15 95. The method of claim 86, wherein the entire library is screened.
96. The method of claim 1, wherein a cloning site is operably positioned in relation to the marker gene.
- 20 97. The method of claim 96, wherein the multiple cloning site comprises at least two restriction sites.
98. The method of claim 96, wherein the multiple cloning site comprises at least ten restriction sites.
- 25 99. The method of claim 96, wherein the multiple cloning site comprises at least one hundred restriction sites.
- 30

100. The method of claim 1, wherein the candidate nucleic acid is cloned into said plasmid by TA cloning.

101. A method of screening candidate nucleic acid for one or more nucleic acid sequences encoding a polypeptide comprising a signal sequence and/or a transmembrane sequence comprising:

- a) providing a bacterial cell;
- b) contacting the bacterial cell with at least one plasmid comprising a candidate nucleic acid segment and a marker gene comprising a mutation in a region comprising a signal sequence and/or a transmembrane sequence of the marker gene; and
- c) screening for function of the marker gene;

wherein function of the marker gene indicates that the candidate nucleic acid segment comprises a sequence that encodes a polypeptide comprising a signal sequence and/or a transmembrane sequence.

102. A method of screening candidate nucleic acid for one or more nucleic acid sequences encoding a polypeptide comprising a signal sequence and/or a transmembrane sequence comprising:

- a) providing a bacterial cell;
- b) contacting the bacterial cell with at least one construct comprising a candidate nucleic acid segment and a mutated selectable marker gene comprising a mutation in a region comprising a signal sequence and/or a transmembrane sequence of the marker gene; and
- c) screening for survival of the cell on selectable media;

wherein survival of the cell or its progeny cells on the selectable media indicates that the candidate nucleic acid segment comprises a sequence encoding a polypeptide comprising a signal sequence and/or a transmembrane sequence.

103. A construct for screening for nucleic acid sequences encoding a polypeptide comprising a signal sequence and/or a transmembrane sequence comprising:

- a) a replication system functional in a bacterial host cell;
- b) at least a first marker gene; and
- c) a candidate nucleic acid sequence;

wherein expression of the marker gene in a bacterial cell indicates that the candidate nucleic acid sequence encodes a polypeptide comprising signal sequence and/or a transmembrane sequence.

104. The construct of claim 103, wherein the first marker gene is a screenable marker gene.

105. The construct of claim 103, where the first marker gene is a scorable marker gene.

106. The construct of claim 103, where the first marker gene is a measurable marker gene.

107. The construct of claim 103, where the first marker gene is a selectable marker gene.

108. The construct of claim 107, where the first marker gene is an antibiotic resistance gene.

109. The construct of claim 108, where the antibiotic resistance gene is an ampicillin-resistance gene.

110. The construct of claim 103, wherein the marker gene is mutated.

111. The construct of claim 103, wherein the construct further comprises a multiple cloning site.

112. The construct of claim 103, wherein the bacterial cell is a gram negative bacterial cell.

113. The construct of claim 112, wherein the bacterial host cell is an *E. coli* cell.

25044605.1